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Twenty-three cases of hemorrhagic fever with renal syndrome were serologicaly diagnosed in rural population of Greece between 1990-1993. The clinical picture of the disease was severe (2 deaths out of 23 cases) and most closely resembled Asian form of the disease than western European form. Additional, the follow up of the renal function of 12 HFRS patients whowed that 3 patients developed chronic renal dysfunction. Serosurvey results of 2165 blood samples taken from apparently healthy residents (farmers, shepherds and woodcutters) from 13 of 54 counties in Greece, showed the percentage of seropositives to be 4,6% with a range from 1,8% to 15,6%. Small mammal survey results suggest that Apodemus flavicollis is the host of the virus.

None of the 470 patients with a disease resembling Crimean-Congo hemorrhagic fever or with other diseases which are including in the differential diagnosis of Crimean-Congo hemorrhagic fever, was found to be infected with CCHF virus. In contrast, results obtained from human and animal serosurveys showed that 1,9% of the individuals examined and 1,4% of the animals had antibodies to CCHF virus.

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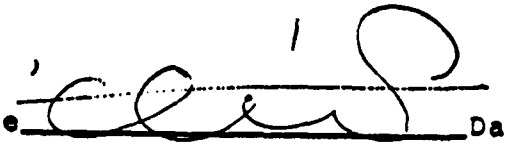
PI Signature  Date 20.12.93

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INTRODUCTION

Viruses of the genus Hantavirus (Bunyaviridae) are zoonotic agents with a global distribution. Virus serotypes are maintained primarily by rodent reservoirs, are they cause a spectrum of human disease across Eurasia referred to collectively as Hemorrhagic Fever with Renal Syndrome (HFRS). Four dinstict hantaviruses are recognized on the basis of serologic differences, and they include Hantaan, Puumala, Seoul, and Prospect Hill viruses. Each of these viruses is primarily associated with a single species of rodent (Apodemus agrarius, Clethrionomys glareolus, Rattus norvegicus, and Microtus prnnsylvanicus, respectively). A fifth virus (Leakey virus) isolated from Mus musculus has been proposed as a new virus type of the Hantavirus genus. Clinical presentation of HFRS is variable, and in geographic regions such as eastern Asia, the Balkans, and western Europe [1].

Crimean-Congo haemorrhagic fever (CCHF) is a widespread arboviral disease that has been reported throughout sub-saharan Africa, central and southern Europe, Asia, and the Middle East. Moreover, CCHF virus (genus Nairovirus; family Bunyaviridae) has been isolated from numerous hosts in a variety of ecosystems. The maintenance of CCHF virus in nature involves several vertebrate hosts and tick vectors that apparently combine to create complex virus cycles. The roles of each host or vector, however, have not been well defined. Tick abundance is strongly correlated with virus transmission to humans and vertebrates. Various vertebrates are able to replicate CCHF virus without clinical symptoms and only humans experience disease. In a review of the clinical syndromes caused by tick-borne arboviruses it was hypothesized that the observed variability pathogenicity of CCHF virus to human in different areas may be more realted to the vector than to the regions per se [2]. Moreover, our compilation of available sources, suggests that the case-fatality rate could vary with the source of infection in Greece [3].

Sofar Hantaan endemic areas have been identified in northern and central Greece [4], the clinical course of the severe form of the disease as it occurs in Greece has been described [5], a hantavirus has been isolated from a severely ill HFRS patient, and the probable rodent host of

the virus has been described [6].

In a previous study, 1025 blood samples (single or paired) from patients with suspected C-CHF or from patients with pyrexia of unknown origin, influenza-like diseases, and pyrexia with elevated liver enzymes (SGOT-SGPT) were sent to this laboratory for serodiagnosis. None of these patients was found to be infected with C-CHF virus. In contrast, C-CHF virus is endemic in Former Yugoslavia, Bulgaria and Albania in areas bordering northern Greece and causes human disease [7, 8, 9]; E. Eltari, pers. comm. 1988].

The proposed work of this study was the case-findings as well as serological testing to determine the antibody prevalence and distribution of CCHF and HFRS, a follow up study of renal function in individuals previously infected by Porogia virus, and isolation of Hantavirus from serum and/or from blood cells from HFRS patients and from rodents. Additionally attempts would be made for CCHF virus isolation from CCHF patients and ticks.

MATERIAL METHODS AND RESULTS

Patients

Five hundred and thirty-two blood samples (single or paired) were collected from acute and convalescent sera for HFRS and CCHF serodiagnosis between 1990-1993 from patients whose illnesses had been clinically diagnosed as haemorrhagic fever with renal syndrome, acute nephritis or acute renal insufficiancy, Leptospirosis, and from patients with influenza like-diseases, pyrexia of unknown origin, pyrexia with elevated liver enzymes and from CCHF suspected patients. Additionally 12 HFRS patients who discharged with normal renal function, were studied on an outpatients basis for a thorough evaluation of the renal function.

Follow up of the renal function in previously diagnosed HFRS patients.

Twelve patients were studied on an outpatients basis. They were asked to collect a 24-h urine sample, wich was sent to the laboratory for total protein excretion, electrolytes, uric acid,

and creatinine measurements. On their arrival the patients were advised to give a urine specimen for microscopic examination, pH measurement, and culture. Blood pressure was measured in the sitting position. Arterial blood was also taken for pH and HCO_3-measurements; whole venous blood for the determination of creatinine, electrolytes, uric acid, total protein, and globulins was also obtained. Urinary comcentrating ability was studied using the protocol of Gyory et al., in which urinary specific gravity and osmolality were determined after administration of 5 units of vasopressin tannate in oil subcutancously. In order to reveal the existence of the incomlete type of renal tubular acidosis (RTA), the acute acid loading test, using the method of Wrong and Davis, was performed in the nonacidotic patients, following administration of 0.1 g NH_4Cl/kg body weight. This should induce a 4 to 5 mmol/L fall in the plasma HCO_3-concentration within 4 to 6 h. The urine pH will remain above 5.3 in type I RTA but will be less than this value, and usually below 5, in normal subjects in whom acidemia stimulates maximal urinary acidification. pH and urinary anion gap were determined in a urine specimen obtained 6 h following the administration of NH_4Cl. Serum anion gap (SAG) was calculated from the equation: SAG (mmol/L) = Na^+ - (Cl^- + HCO_3^-). Urine anion gap, which in cases of hyperchloremic (normal anion gap) metabolic acidosis is an indirect measure of the quantity of urinary NH_4^+, was calculated from the equation: Urine anion gap (mmol/L) = $Na^+ + K^+ - Cl^-$, either from 24-h urine electrolytes or from urine electrolytes after NH_4Cl loading. Percentage tubular reabsorption of phosphate (% TRP) was calculated from the formula: TRP = 1-Cpo/Ccp were Cpo4 represents renal clearance of phosphate and Ccr represents creatinine clearance. Tm_{po}/GFR (maximum tubular reabsorption rate for phosphate) was calculated from a no-morgam using serum phosphate concentration and % TRT. Electrolytes in sera and urine were determined by flame photometry, and creatinine by the method of Hare. Urice acid was determined by a uricase method and proteinuria was measured by a biuretic method. The Fisher exact test was used for statistical analysis.

Human serosurvey

Sera from 2,165 individuals, mainly farmers, wood-cutters and shephards, were obtained in 14 of the 54 counties of Greece: 10 in Northern Greece (Trace, Macedonia and Epirus States), 1 in Central Greece and 3 in Aegian sea islands. (Mitilini, Chios, Samos). Sera were collected annually from 1990 to 1993 and stored at -20° C until they could be tested for anti-Hantaan and anti Crimean Congo hemorrhagic fever viruses antibody by IFA and ELISA tests. These sera as well as the blood samples collected from patients, were identified by age, sex, occupation, previous travel history (mainly abroad) and residence of the donor.

Animal serosurvey

Sera from 1399 goats (651 sera), sheep (400 sera) and cattles (348 sera) were obtained in 4 counties of Northern Greece (3 in Macedonia state and 1 in Epirus state). Sera were collected annually from 1990 to 1993 and stored at -20°C untill the could tested for anti-Crimean Congo haemorrhagic fever virus antibody by IFA and ELISA tests.

Small mammal collection

Four hundred and seventy-six small mammals were live-trapped in villages and in the fields and forests surrounding 5 villages in which HFRS cases had been previoulsy diagnosed. Whole blood was espirated from mice with capillary tube Pasteur pipettes following rupture of the retro-orbital sinus. Rats were anesthetized with 0.1 ml intramuscular infection of Ketamine, then exsanguished by cardiac puncture and the obtained sera were kept at -20°C untill they used for anti-Hantaan antibody by IFA test. Lungs, spleen and kidney were aseptically removed and stored individually in plastic vials containing liquid nitrogen untill they used for Hantaan virus isolation. Additionally, captured animals were identified by species.

Tick collection

On thousand and five-hunderd ticks were collected from the body of animals (goat, sheep

and cattles) in 5 counties of Northern Greece, **(Table 12)**. After identification in species, 142 pools were assembled to contain 5 to 15 living ticks of the same species. Date and place of collection were identified. Pools were stored at -70°C untill they used.

Methods

Serological techniques

Patient's sera, apparently healthy individuals' sera and animal sera collected for serosurvey as well as small mammals sera, were examined by IFA and ELISA tests with the corresponding conjugated immunoglobulins (goat anti-human, rabbit anti-goat, and rabbit anti-mouse, fluorescence and peroxidase conjugated immunoglobulins). For spot-slides, Vero E-6 cells were infected with Hantaan viruses (strains Porogia, Puumala and Urban Rat) and Crimean-Congo hemorrhagic fever infected cells were harvested on day 7, mixed with uninfected cells and 50 μl of the mixture was dropped on to each spot on the spot-slide. The spot-slides were incubated at 37° C overnight for cell attachment and growth. Next, the mixture was dried as spot-slides. Spots with uninfected cells were used as controls. About $3x10^3$ cells constituted each spot. The slides were fixed in acetone and stored frozen. For antibody detection, slides were overlayed with human and animal sera, washed and stained with FITC-congugated antihuman IgG and IgM or antigoat IgG and IgM. For surveys sera were considered as positive if characteristic fluorenscence was detected at 1:8 and 1:32 for Crimean-Congo hemorrhagic fever and Hantaan viruses respectively. A 4-fold or greater rise in titer between acute and cenvalescent phase sera of IgG antibody and/or a high titer of IgM antibody (≥1:128) were taken as current infection.

The Enzyme-Immunoabsorbent (ELISA) assay test was applied for antibody detection as well as serodiagnosis for both Crimean-Congo haemorrhagic fever and Hantaan virus human infections. Antigens for both Crimean-Congo haemorrhagic fever and Hantaan viruses were prepared from the growth media of infected Vero E-6 cells with Crimean-Congo hemorrhagic fever and Hantaan viruses. Growth media was centrifuged at 3.000 rpm and the supernantant was dialysed, and

filtered through a 440 micron membrane and tested in the ELISA with positive serum to determine the optimal dilution. In parallel negative antigen control from univfected Vero E-6 cells was prepared. The ELISA was done in Dynatech 96-well microelisa plates. Antigen was attached to the solid phase and coating was carried out overnight at 4°C. Bovine albumin 0.5% blocking was utilized. Human as well as animal sera was added, incubated 1 hour at 37°C and washed. The alkaline phosphate conjugated antihuman or antigoat immunoglobulin was then added, incubated 1 hour at 37°C, washed, and exposed to p-nitrophenyl phosphate substrate. Three negative human or animal sera were included in each test. Three times the SD of the average OD of these plus the OD of the negative antigen substracted from that of the positive will constitute a positive reaction if greater than O. ELISA antigen capture test and IgM capture assays were applied for the detection of CCHF antigen or IgM antibody in the sera HFRS and CCHF suspected patients.

Virus isolation

Hantavirus isolation from whole blood and lymphocytes of HFRS patients.

Whole blood was obtained during the acute phase of the disease from 14 HFRS patients. One-milliliter aliguots of the blood were inoculated onto Vero E-6 cells grown in 25-cm^2 plastic flasks. Inoculated flasks were incubated at 37°C for 15 days, then the cells were suspended with trypsin and passed to fresh flasks. While suspended, some cells were used to prepare 10 well spot-slides, which were then fixed in cold acetone and examined for characteristic hantavirus antigen by IFA test with human antibody to Hantavirus. Blind passages were continued for 60 days with examination for the virus at 15 days intervals.

For Hantaan virus isolation from lymhocytes, whole blood was obtained during the acute and convalence phase of the disease from 12 HFRS patients. Lymphocytes were separated from fresh 10 ml whole blood on Ficoll-Hypaque gradients and were placed into culture with PHA, incubated for 72h at 37°C with 5% Co2. Then $1,5x10^3$ PHA-stimulated lymphocytes were co-cultivated with Vero E-6 cells in 25cm^2 plastic flasks and incubated at 37°C with 5% CO2. While

co-cultivated some PHA-stimulated lymphocytes were used to prepare 10-well spot-slides, which were then fixed in cold acetone and examined for characteristic hantavirus antigen. Weekly passages were continued for 7 weeks and in every passage PHA was added.

Hantaan virus isolation from small mammals

Lung tissues from 13 seropositive and 13 seronegative rodents were dissociated with a mechanical blender. One hundred milliliters of suspension were inoculated onto Vero E-6 cells grown in 25-cm^2 plastic flasks. Inoculated flasks were incubated at 37°C for 15 days, then the cells were suspended with trypsin and passed to fresh flasks. While suspended, some cells were used to prepare 10 well spot-slides, which were then fixed in cold acetone and examined for characteristic hantavirus antigen by IFA assays with human antibody to Hantaan virus. Isolation attempts were considered negative if characteristic hantavirus antigen was not detected by IFA after 60 days of serial, blind passage.

Crimean Congo haemorrhagic virus isolation from ticks

One hundred forty-two tick pools were ground individually in a mortan and fluid was added to produce a suspension approximately 10%. All dilutions were made in phosphate buffered saline, pH 7,2 containing 1% bovine albumin (fraction V), penicillin (1000 units per ml) and streptomycin (1mg per ml). After light centrifugation (3000 rpm x10') 1 ml of supernate was inoculated onto Vero E-6 and CER cells grown in 25-cm^2 plastic flasks. Inoculated flasks were incubated at 37°C for 15 days. Then, the supernate was kept at -70°C and the cells were used to prepare 10-well spot-slides, which were then fixed in cold acetone and examined for characteristic Crimean Congo haemorrhagic fever virus antigen by IFA assays with human and mouse antibody to Crimean Congo Hemorrhagic fever virus.

RESULTS

HFRS patients, and follow up of the renal function of HFRS patients

Five hundred and four male and female farmers, shepherds and woodcutters were admitted to various hospitals of Thessaloniki and other hospitals of Northern Greece, with clinical diagnosis of HFRS, Leptospirosis, acute renal insufficiency, influenza like diseases and pyrexia of unknown origin. The diagnosis of HFRS was serologically confirmed in 23 HFRS suspected patients, in one with clinical diagnosis of Leptospirosis and in one with pyrexia of unknown origin by rising antibody titers (IgM and IgG) to Hantaan virus. None of the 102 patients with influenza-like diseases was found to be infected by Hantaan virus **(Table 1)**. Analysis of data concerning clinical signs and symptoms and laboratory data of the disease as reported in patient's medical records is shown in **(Table 2)**. Of 23 serologically diagnosed cases, 2 (8%) died and 10 (44%) developed severe symptoms including flushing over face, and neek, conjuctival injection, pneumonic infection, pulmunary edema, confusion, shock and hemorrhagic manifestations. Five of these patients (21%) required renal dialysis. The predominant symptoms in all patients were fever, headache, neusea, vomiting and abdominal pain, proteinuria with microscopic hematuria, and increased serum urea and creatinine were present in all patients. In patients with hemorrhagic manifistations, the platelet counts were generally $< 10^5$ cells/μl.

Follow up the renal function of HFRS patients

All the examined individuals were normotensive and had creatinine clearance within the normal limits for their age (between 90 and 120 mL/min). None had albuminuria or glucosuria. However, 3 out of them had renal tubular acidosis type I (distal): One out of these 3 patients had the complete type of the disease with hyperchloremic metabolic acidosis, normal serum anion gap, increased urine pH and anion gap, and mild hypokalemia with inappropiate kaliura. The other 2 patients had the incomplete type of distal RTA type I. These patients had normal arterial pH under normal conditions but exhibited defective urinary acidification after an acid load test, defined as an increased urine anion gap as well as an increased (>5.5) urine pH **(Table 3)**. Two

patients (1 of whom had also incomplete RTA) had diminished urinary concentrating ability. These patients had decreased U_{osm} which was not increased appropriately after administration of vasopressin **(Table 4)**. Serum levels of uric acid and phosphorus were within normal limits and there was no inappropriate uricosuria (urine uric acid/urine creatinine <1 and 24-h urine uric acid <800 mg for men and <750 mg for women) or phosphaturia (TRP >80% and Tm_{po}/GFR >2.5) in all examined individuals. No association was found between the severity of the acute illness-defined as heavy proteinuria, increased serum creatinine, severe thrombocytopenia, and raised transaminases-and the development of chronic renal dysfunction **(Table 5)**.

Crimean Congo hemorrhagic fever

None of the CCHF and Leptospirosis suspected patients, and patient with influenza like diseases, pyrexia of unknown origin and pyrexia with elevated liver enzymes with found to be infected with CCHF virus **(Table 6)**.

Human serosurvey

Hantaan virus

Two thousand and one hundred sixty-five human sera collected from 13 counties were examined by IFA and ELISA tests during the grant period. The overall antibody prevalance rate was 4,4% and 4,8% for IFA and ELISA methods respectively **(Table 7)**. All sera analyzed together and the results summarized according to region and county of origin in which the serosurveys were conducted as shown in **(Table 8)**. The overall antibody prevalence rate was 4,6 with a range from 1,8 to 15,6%. The age distribution is shown in **(Table 9)**. Young individuals (up to 30 years) were found to be infected with Hantaan virus and the ratio of infected males to infected females was approximately 3:1. Seropositives were detected in all counties. The first cases of the disease appeared in late April and cases were observed until late October. The highest risk group ranged from 35 to 50.

Crimean Congo hemorrhagic fever virus

The same human sera that examined for antibody to Hantaan virus were examined also for antibody to Crimean-Congo hemorrhagic fever virus. The overal antibody prevalance rate was 0,96% and 1,66% for IFA and ELISA tests respectively **(Table 7)** with a range from 0-14,7% **(Table 8)**. Seropositive were detected in 11 of 13 counties **(Table 8)**. The age distribution is shown in **(Table 9)**. Middle-age individuals (41 to 50) were found to be infected with Crimean-Congo hemorrhagic fever virus and the ratio of infected males to infected females was approximately 2:1. Seropositives were detected in 11 of 14 counties.

Animal and small mammal survey

Hantaan virus small mammal survey

During the grant period 476 small mammals were collected from 6 endemic areas (Ioannina, Kilkis, Kastoria, Drama, Imathia, Serres). Apodemus flavicollis was common in fields in these areas. High IFA antibody titers were detected in 12 Apodemus flavicollis sera and in one Mus musculus **(Table 10)**.

Crimean-Congo hemorrhagic fever animal survey

One thousand and three hundred ninty-nine goat (651 samples), sheep (400 samples), and cattle (348 samples). Sera were collected from 4 counties where human seropositives were found (Ioannina, Kilksi, Drama, Kastoria). Antibody to Crimean-Congo hemorrhagic fever virus were found in goats (1,8%) and in sheep (1,0%) whereas none of the cattles examined was found positive **(Table 11)**. Also the highest prevalence of seropositive goat was found in goats collected in Kastoria county where the human seropositive prevalence is the highest in Greece.

Virus isolation

Hantaan virus isolation from humans, and small mammals

All attempts to isolate virus from HFRS patients blood and from lung tissues of Apodemus flavicollis have been unsuccessful. Hantaan virus antigen was detected in 4 patients lymphocytes but the recovery of the virus from the lymphocytes was unsuccessful.

Crimean-Congo hemorrhagic fever virus from ticks.

All attempts to isolate Crimean-Congo hemorrhagic virus from ticks pools have been unsuccessful.

CONCLUSIONS:

Hemorrhagic fever with renal syndrome

HFRS is endemic in Greece and the disease is much more severe than nephropathia epidemica in Scandinavia and western Europe. Despite of the rapid clinical and serological diagnosis as well as proper symptomatic treatment in specific hospital units, 2 out of 23 patients died (8%) and among the 23 cases studied 11 were severerly ill, and 4 required renal dialysis. Additionaly shock, pulmunary edema, and hemorrhagic manifestations developed in 5, 3, and 5 patients respectively. The clinical findings from Greek patients suggest that the disease is more like the severe Asian form of HFRS caused by Hantaan virus than the milder western European form and nephropathia epidemica caused by Puumala virus. In southeastern Europe, Porogia virus (Greek isolate) and Belgrade viruses (Former Yugoslavia isolate) are endemic causing severe disease in humans, in this area of Europe.

The follow up of the renal function in 12 patients with HFRS showed that 3 had renal tubular acidosis (RTA) type 1, complete or incomplete and 2 had recuced urine concentrating ability. Concluding that HFRS causes chronic renal dysfunction in a limit number of patients.

Results from the serosurvey screening and in the eastern islands of Aegian sea (Mitilini, Chios, Samos), revealld that the virus is spread throughout the country and further on, suggest

the possible existance of the virus in Minor Asia (Turkey).

Crimean Congo hemorrhagic fever

Results obtained from the serodiagnosis of CCHF and Leptospirosis suspected patients as well as patients with influenza like diseases, patients with pyrexian of unknown origin with elevated liver enzymes releaved that none of these patients was found to be infected with CCHF virus. In contrast, antibody to CCHF virus were found in animal and in individuals who had no recollection of an illness clinically ressembling a serious type of Crimean hemorrhagic fever. Considering that a CCHF virus (strain AP92) was isolated in Greece 1976 it seems that CCHF human infections occur in Greece but the infection does not result on a serious disease or even more the infection could be inapparent.

The unssuccesful virus isolation from ticks maybe due to the use of Vero E-6 and CER cells instead of suckling mice.

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Table 1: Serological diagnosis by IFA and ELISA of HFRS supected patients, Leptospirosis, suspected patients, patients with Influenza like diseases and patients with pyrexia of unknown origin.

Suspected disease	No of patients	Serological diagnosis
HFRS	186	21
Leptospirosis	97	1
Influenza-Like	102	0
Pyrexia of unknown origin	119	1
TOTAL	504	23

Table 2: Clinical symptoms signs and laboratory data in 23 Greek patients with HFRS.

Symptoms	No of patients	Signs (findings)	No of patients
Fever	23	Renal insufficiency	23
Rigors	20	Haemorrhage	14
Malaise	19	Flushing (face and neck)	12
Myalgias	19	Conjuctival injection	12
Headache	17	Hypotension	12
Vomiting	16	Confusion, precoma or coma	7
Backache	14	Shock	7
Arthralgias	8	Pulmonary infiltrates	4
Diarrhoea	5	Pulmonary edema	2
Abdominal pain	18		

Proteinuria (ranging from trace to >2g/24h	138
Haemamaturia (6-10 red blood cells/h.p.f.)	138
WBC (cells/μl) > 9.000	12
Haematocrit >52%	11
Platelets < 100.000 cells/μl	7
Platelets (100.000-150.000 cells/μl)	6

Table 3: Laboratory Parameters of the 3 Patients with Impaired Acidification

Patient	Arterial pH	Serum Cl^- (mmol/L)	Serum K^+ (mmol/L)	Serum HCO_3 (mmol/L)	Serum anion gap (mmol/L)	Urine pH	Urine K^+ (mmol/24h)	Urine anion gap (mmol/L)
1	7.29	110	3.4	19	11	5.9	32	64
2	7.35(7.29)	102(109)	3.8(3.6)	23(19)	10(11)	6.0(5.8)	42	(56)
3	7.38(7.30)	101(104)	3.8(3.6)	24(20)	9(10)	6.1(5.8)	44	(48)

Values in parenthesis obtained after NH_4Cl loading.

Calculated from the 24-h urine electrolytes.

Calculated from urine specimen received 6 h after NH_4Cl loading.

Table 4: Urinary Concentration Test

	Specific gravity		Uosm (mOsmol/kg H_2O)	
	Before vasopressin	After vasopressin	Before vasopressin	After vasopressin
Patients with diminished renal concentrating ability (n=2)	1.007±0.002	10.11±0.002	280±30	420±50
Patients with normal renal concentrating ability (n=10)	1.016±0.003	1.024±0.003	510±40	810±60

Table 5: Association of Chronic Renal Dysfunction with the Severity of Acute Illness

	Patents with chronic renal dysfunction (n=6)	Patients without chronic renal dysfunction (n=8)
Severe proteinuria >3.5 g/24h	1/6	1/8
Increased serum creatinine >8mg/dL	2/6	4/8
Severe thrombocytopenia PLT<30.000/mm^3	3/6	3/8
Raised serum transaminases >100 IU/L	2/6	3/8

Including the 2 patients who were discharged with some degree of renal impairment (see Introduction).

Table 6: Serological diagnosis by IFA and ELISA tests of CCHF suspected patients, Leptospirosis suspected patients, patients with influenza like disease, with pyrexia of unknown origin and patients with pyrexia and elevated liver enzymes.

Suspected disease	No of patients	Serological diagnosis
CCHF	28	0
Leptospirosis	97	0
Influenza-Like disease	102	0
Pyrexia of unknown origin	111	0
Pyrexia with elevated liver enzyme	132	0
TOTAL	470	0

Table 7: Antibody to Crimean-Congo haemorrhagic fever and Hantaan viruses in healthy residents of Greece.

Virus	No tested	No positive	%	Method
Crimean-Congo haemorrhagic fever	2165	21	0.96%	IFA
Crimean-Congo haemorrhagic fever	2165	36	1.66%	ELISA
Hantaan	2165	97	4.4%	IFA
Hantaan	2165	105	4.8%	ELISA

Table 8. Antibody to Crimean-Congo haemorrhagic fever and Hantaan viruses in healthy residents of Greece: geographic distribution.

State / Country	Hantavirus		Crimean-Congo haemorrhagic fever virus	
	Total tested	% positive	Total tested	% positive
Thrace				
Rodopi	165	1.8	165	0.6
Evros	140	2.8	140	0.7
Macedonia				
Drama	155	1.9	155	0.6
Serres	148	3.3	148	1.32
Kilkis	162	3.7	162	1.8
Imathia	145	8.2	145	1.3
Kastoria	168	4.7	168	1.7
*Grammos	102	15.6	102	14.7
Florina	171	5.2	171	2.3
Central Greece				
Tricala	197	3.0	197	0.0
Epirus				
Ioannina	186	8.0	186	2.1
Aegians sea islands				
Mitilini	103	1.9	103	0.0
Chios	205	2.4	205	0.4
Samos	118	2.5	118	0.0

* Grammos area is located in Kastoria county.

Table 9: Antibody to Crimean-Congo hemorrhagic fever and Hantaan viruses in healthy residents of Greece, by age.

Age (years)	Crimean-Congo hemorrhagic fever No (%)	Hantaan No (%)		
0-10	42 (0.0)	42	1	(2.3)
11-20	215 (0.0)	215	5	(2.3)
21-30	283 (0.0)	283	16	(5.6)
31-40	312 (0.3)	312	21	(6.7)
41-50	512 (0.9)	512	23	(4.7)
51-60	476 (0.6)	476	13	(2.7)
61-70	232 (3.4)	232	11	(4.7)
>70	93 (4.3)	93	7	(7.5)
Total	**2165**			

Table 10 : Small mammals captures in HFRS endemic areas and tested by IFA test for antibody to Hantavirus.

Genus/species	No of trapped rodends	Seropositive	IFA Titers
Rattus rattus alexandrinus	83	0	-
Rattus rattus frugivoris	31	0	-
Apodemus flavicollis	156	12	1:64-1:1024
Apodemus sylvaticus	58	0	-
Crocidura sp.	15	0	-
Mus musculus	133	1	1:64
TOTAL	476	13	

Table 11: Animal survey for antibody to CCHF virus

Animal species	Area (county)	No tested	No of positive
Goat	Ioannina	182	2
Goat	Kilkis	145	2
Goat	Drama	108	1
Goat	Kastoria	216	12 (1,8%)
Sheep	Ioannina	153	1
Sheep	Kilkis	85	0
Sheep	Kastoria	162	4 (1,0%)
Cattle	Ioannina	97	0
Cattle	Kilkis	110	0
Cattle	Kastoria	141	0
TOTAL		1399	16

Table 12: Tick pools from Northern Greece (mainly Macedonia) examined for CCHF virus isolation in Vero E-6 and CER cells.

Tick species	Number of pools	Animals species
Ixodes ricinus	10	Goat, sheep
Ixodes gibbosus	19	Goat, sheep
Rhipicephalus bursa	37	Goat, sheep, cattle
Rhipicephalus sanguineus	28	Goat, sheep, cattle
Hyalomma anat. anatolicum	17	Goat, sheep, cattle
Hyalomma marg. marginatum	12	Sheep, cattle
Hyalomma maur. mauritanicum	3	Goat, cattle
Dermacentor marginatus	11	Goat, sheep, cattle
Haemaphyalis leachi	1	Goat
Boophilus calcaratus	2	Cattle
Boophilus calc. balcanicus	2	Cattle
TOTAL	142	